

PARTHENOGENETIC MEROGONY OR DEVELOPMENT
WITHOUT NUCLEI OF THE EGGS OF SEA
URCHINS FROM NAPLES

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In a previous paper (1936) it was shown that non-nucleate parts of the eggs of *Arbacia punctulata*, obtained by centrifugal force, can be artificially activated and by successive cleavages they reach the blastula stage. This I have termed parthenogenetic merogony since it is a combination of parthenogenesis, in which the male nucleus is lacking, and merogony, in which the female nucleus is lacking; in parthenogenetic merogony both male and female nuclei are lacking, and the egg develops without any nuclei. In view of the fact that the non-nucleate halves of *Arbacia punctulata*, obtained by centrifugal force, are densely packed with pigment granules which apparently interfere somewhat with the formation of typical cell boundaries, even after fertilization, it seemed possible that further development of parthenogenetic merogones might be obtained with other species of sea urchin. Furthermore, the presence of mitochondria in the non-nucleate halves of certain species presented a means of testing the possibility that these granules might be helpful in development without nuclei. An investigation was carried out at Naples in the spring of 1937 and the results of the study are presented in this paper. Some observations on parthenogenetic and merogonic development are included, to serve as a basis for comparison of eggs without any nucleus (parthenogenetic merogones) with similar eggs and egg fractions having one nucleus, female or male (= "haplones"). A study has previously been made (1933) of the development of the centrifuged whole eggs and their nucleate fractions after fertilization, that is with two nuclei, both female and male (= "diplones"). Some observations on the effect of activating agents on immature eggs are also included since these bear on the general subject.

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MATERIAL AND METHOD

The four species of sea urchins commonly occurring at Naples are *Arbacia pustulosa*, *Sphaerechinus granularis*, *Paracentrotus* (*Strongylocentrotus*) *lividus* and *Parechinus* (*Echinus*) *microtuberculatus*. The eggs of all these species can be broken apart by centrifugal force and these parts can be fertilized and will develop (1933). The nucleus always goes to the light pole, so that the heavy half of the egg is always non-nucleate. With further centrifuging, the halves can be broken into quarters. The non-nucleate fractions of the eggs of all four species can be artificially activated. The egg of *Parechinus microtuberculatus* proved the best one for the investigation since it breaks apart easily (thus avoiding heating while on the centrifuge) and the very clear protoplasm renders all phenomena easily visible in the living egg.

The most satisfactory parthenogenetic agent was the same as was used for *Arbacia punctulata*, i.e., sea water concentrated to about half its volume, or NaCl added to the sea water to bring it to a similar hypertonicity. After immersion for 10–20 minutes, the eggs were returned to sea water. Other methods were tried, especially Loeb's double method (butyric acid and a hypertonic solution) with less favorable results. One difficulty in the investigation has been to obtain a standard concentration and a definite period of immersion in the hypertonic solution owing to the extreme variability in reaction of different batches of eggs of the same species. Such has been the experience also of other investigators in obtaining parthenogenesis of entire eggs.

STRATIFICATION BY CENTRIFUGAL FORCE

When the eggs of any of the four species are centrifuged, the nucleus always goes to the light pole under the oil cap. This is invariable in mature unfertilized eggs. The stratification of materials, however, is different in the different species, thus causing a difference in granular content of the non-nucleate fractions of the different species; these fractions also differ in size in comparison with their corresponding nucleate fractions. The stratification of the four species is shown in Photographs 1–13.

The normal egg of *Arbacia pustulosa* is exactly like that of *Arbacia punctulata* except that it is a little larger ($79\ \mu$ as vs. $74\ \mu$) and more heavily pigmented; and it stratifies similarly. The stratification of *Arbacia pustulosa* (Photograph 1) is:—oil at the light pole, then a clear layer, then a band of mitochondria (small granules which stain purple with methyl green), then yolk granules, and then red pigment granules at the heavy pole. In the other three species pigment granules are

lacking, except for the faint (occasionally quite reddish) band encircling the *Paracentrotus* egg, which seems to be a definite structure not moved by the centrifugal forces used in my experiments. This is probably due to the fact that the pigment granules forming the band are embedded in the cortical protoplasm which is generally conceded to have a higher viscosity than the interior. The egg of *Arbacia pustulosa* breaks apart in 3 minutes at $10,000 \times g$ into two almost equal halves, the non-nucleate half containing only yolk and pigment and no mitochondria (Photograph 2). With further centrifuging, the halves can be broken into quarters (Photograph 3).

The normal egg of *Sphaerechinus granularis* averages 98μ in diameter. The stratification under centrifugal force is similar to that of *Arbacia* except for lack of pigment granules; i.e., oil, clear layer, mitochondria and yolk (Photograph 4). There is frequently a clear layer also at the centrifugal pole below the yolk (Photograph 5). The egg breaks apart at $10,000 \times g$ in 5 minutes (Photograph 6). The non-nucleate fraction is much larger than the lighter nucleate fraction and contains yellowish yolk granules and a few mitochondria. With further centrifuging both half-eggs can be broken into quarters.

The eggs of the other two species differ from those of *Arbacia* and *Sphaerechinus* in having the mitochondria heavier than the yolk granules. The egg of *Paracentrotus lividus* has an average diameter of 90μ . The usual stratification under centrifugal force is:—oil, clear

PHOTOGRAPHS 1-13

Stratification and Breaking of Eggs

The photographs were all taken of the living eggs with a Leica camera. Photographs 1, 4, 5, 6, 7, 12 were taken of eggs in a centrifuge microscope slide immediately after being rotated on the centrifuge microscope, and all with the same magnification, so that the sizes of the eggs as pictured are comparative.

PHOTOGRAPH 1. *Arbacia pustulosa*—Stratification.

PHOTOGRAPH 2. *Arbacia pustulosa*—Stratified whole and half-eggs.

PHOTOGRAPH 3. *Arbacia pustulosa*—Red half and its two quarters.

PHOTOGRAPH 4. *Sphaerechinus granularis*—Stratification.

PHOTOGRAPH 5. *Sphaerechinus granularis*—Stratification showing clear layer at bottom of eggs.

PHOTOGRAPH 6. *Sphaerechinus granularis*—Breaking apart.

PHOTOGRAPH 7. *Paracentrotus lividus*—Usual stratification.

PHOTOGRAPH 8. *Paracentrotus lividus*—Stratification with clear layer at bottom of egg.

PHOTOGRAPH 9. *Paracentrotus lividus*—No clear layer at top of egg (Like *Parechinus*).

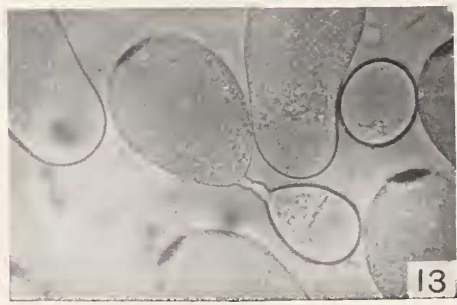
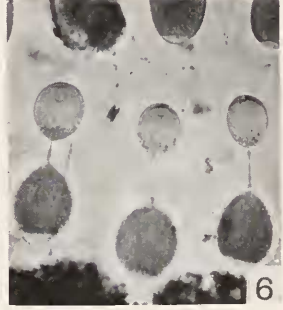
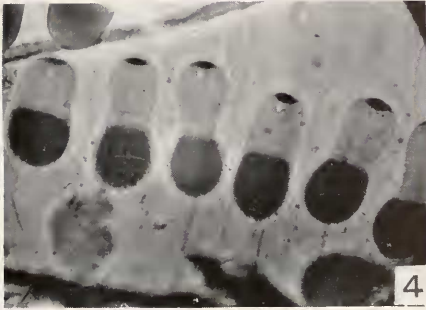
PHOTOGRAPH 10. *Paracentrotus lividus*—Breaking into large upper and small lower halves.

PHOTOGRAPH 11. *Paracentrotus lividus*—Breaking into small upper and large lower halves.¹

PHOTOGRAPH 12. *Parechinus microtuberculatus*—Stratification.

PHOTOGRAPH 13. *Parechinus microtuberculatus*—Breaking apart.

PLATE I



layer, yolk, clear layer and mitochondria (Photograph 7). There is, however, some variation in the position of the clear layer; in some eggs there is no clear layer between the yolk and mitochondria; in some eggs there is a clear zone below the mitochondria (Photograph 8). And sometimes there is no clear layer below the oil (Photograph 9); in this case the stratification is like that of *Parechinus* (Cf. Photographs 9 and 12). The clear substratum is apparently, therefore, not uniform, but must be of graded density throughout its extent in the centrifuged egg. It takes 30 minutes at $10,000 \times g$ to break these eggs apart; they are much more difficult to break apart than the eggs of the other species. There are two types of breaking. The egg may break into a larger nucleate fraction and a small non-nucleate sphere, as the majority of eggs did in the springs of 1933, 1934 and 1937 (Photograph 10) or they may break into a smaller nucleate fraction and a large non-nucleate fraction as the majority of eggs did in 1932 (Photograph 11) (E. B. Harvey, 1933). The two types must be due to something inherent in the egg as they may occur in eggs from the same female centrifuged together in the same tube, although any set of eggs tends to be either of the one type or the other. A similar peculiarity has been noted previously for *Arbacia punctulata* in unusual batches of eggs (1936). In either case, the non-nucleate sphere of *Paracentrotus* contains all the mitochondria and some of the yolk granules.

The egg of *Parechinus microtuberculatus* is the largest of the four species; its average diameter is 102μ . The typical stratification is:—oil, yolk granules, clear layer, mitochondria (Photograph 12). The nucleus, therefore, lies typically among granules at the centripetal pole and not in a clear layer as in the eggs of the other species of sea urchins and of most other eggs. There is occasionally, however, also a clear layer under the oil in which case this egg resembles the typical *Paracentrotus* egg. The egg breaks apart in 8 minutes at $10,000 \times g$, and with further centrifuging the lower half can be broken into quarters. The non-nucleate halves are much smaller than the nucleate, and contain mitochondria and practically no other granules (Photograph 13).

We see, then, that the eggs of the four species of sea urchin under consideration stratify differently, and that therefore the non-nucleate fractions contain different kinds of granules. In the non-nucleate halves of *Arbacia*, pigment granules are present, in the other three species, they are not. In *Sphaerechinus*, *Paracentrotus* and *Parechinus*, mitochondrial granules are present, in *Arbacia*, they are not. In *Parechinus* there are practically no yolk granules, but these are present in the non-nucleate halves of the other three species. The clear protoplasm which is the matrix in which granules are suspended is, of

course, present in all the non-nucleate halves, in greater or less amount, and appears as a definite layer when free of granules, as is typical for *Parechinus*.

ARBACIA PUSTULOSA

Parthenogenetic Merogones

The non-nucleate red halves, yolk quarters and pigment quarters of *Arbacia pustulosa* have been activated artificially (= parthenogenetic merogones). They throw off a fertilization membrane closely adhering to the surface and form a thick ectoplasmic layer, as is characteristic of the normal fertilized egg of this species. A large monaster appears in many of the fractions about two hours after activation, succeeded in another hour or so by an amphiaster; these are usually visible only in the yolk quarters and the less pigmented part of the red halves. After another hour, a cleavage plane may come in between the two asters. This has been observed to come in chiefly in the long axis of the elongate red half-eggs and to extend only to the pigmented area. Some of the red halves have reached by successive cleavages a 16-cell stage with good cell boundaries. Photographs 14–17 are successive photographs of the same two eggs. In many red halves, cell boundaries do not come in, but the egg may be literally peppered with small asters (Photograph 18). In many of the red half-eggs, a cleavage takes place at the pigmented end (Photographs 19, 20). In the yolk quarters which are usually spherical, the first cleavage plane divides the fraction equatorially or nearly so (Photograph 21). No further development has been obtained of the parthenogenetic merogones of *Arbacia pustulosa*, but it seems likely that with a more extensive study, blastulae could be obtained similar to those of *Arbacia punctulata*.

Haplones

The term "*haplone*" is used to include eggs which develop with only one nucleus whether female (= parthenogenetic) or male (= merogonic, i.e. a fertilized egg fragment from which the female nucleus has been removed) and therefore containing a haploid set of chromosomes. The normal fertilized egg, containing both male and female nuclei and therefore a diploid set of chromosomes, is termed a "*diplone*."

The normal egg of *Arbacia pustulosa* may be activated by parthenogenetic agents, and also the whole egg after it has been centrifuged, as well as the white half which contains the nucleus. They all throw off a closely adherent fertilization membrane characteristic of the normal fertilized egg. The cleavage is similar to that of the fertilized egg except that it takes place at a slower rate. The first cleavage of the artificially activated egg, both normal and centrifuged, takes place only

after 3 hours (at 20° C.), often much longer, whereas that of the fertilized egg takes place in $1\frac{1}{3}$ hours. The two-cell stage of the elongate centrifuged egg, both fertilized and parthenogenetic, is similar to that of *Arbacia punctulata*; the first cleavage plane usually comes in across the short axis of the egg, in the less dense granular area, dividing the egg into a smaller cell with few granules and a larger pigmented cell (Photograph 22). The first cleavage plane of the spherical white half, both fertilized and parthenogenetic, is equatorial (Photograph 23). Parthenogenetic whole eggs, both normal and centrifuged, and parthenogenetic white halves have given swimming blastulae, but were carried no further.

The non-nucleate halves and quarters when fertilized (= fertilized merogones) have sometimes cleaved quite regularly, when spherical, but at a slow rate (Photograph 24); but often, as in *Arbacia punctulata*, cell boundaries fail to come in, giving rise to multi-nucleate eggs. Nuclei are often plainly visible in these fertilized merogones (Photograph 25). They contrast strikingly in this respect with the parthenogenetic merogones in which asters may be present but not nuclei. A few swimmers have been obtained but many of the fertilized merogones fail to develop.

PARECHINUS MICROTUBERCULATUS

Parthenogenetic Merogones

The non-nucleate halves of *Parechinus microtuberculatus* consist of mitochondrial granules and clear layer (Photograph 26). When they

PHOTOGRAPHS 14-25

Arbacia pustulosa

PHOTOGRAPHS 14-21. Parthenogenetic merogones. Photographs 22-25. Haploes.

PHOTOGRAPH 14. Two red half-eggs, $4\frac{1}{2}$ hours after activation. Two asters were plainly visible in the egg at left.

PHOTOGRAPH 15. Same, 5 minutes later. Cleavage between the two asters.

PHOTOGRAPH 16. Same, 1 hour later ($5\frac{1}{2}$ hours after activation). Note cleavage plane appearing in egg at right.

PHOTOGRAPH 17. Same, $1\frac{1}{2}$ hour later (6 hours after activation).

PHOTOGRAPH 18. Red half-egg, peppered with asters ($7\frac{1}{2}$ hours after activation).

PHOTOGRAPH 19. Red half-egg, $5\frac{1}{4}$ hours after activation.

PHOTOGRAPH 20. Similar red half-egg, 22 hours after activation.

PHOTOGRAPH 21. Yolk quarter, $4\frac{1}{2}$ hours after activation. The cleavage plane was observed to come in between two large asters.

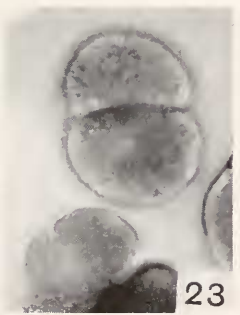
PHOTOGRAPH 22. Whole egg centrifuged, then activated, 6 hours after activation.

PHOTOGRAPH 23. White half-egg, 6 hours after activation.

PHOTOGRAPH 24. Fertilized red half, $5\frac{1}{4}$ hours after fertilization.

PHOTOGRAPH 25. Fertilized yolk quarter, $9\frac{1}{2}$ hours after fertilization. Observe nuclei in the cells.

PLATE II



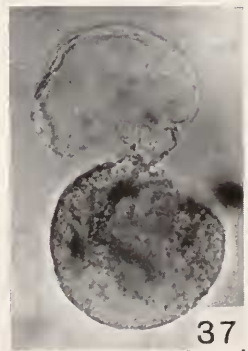
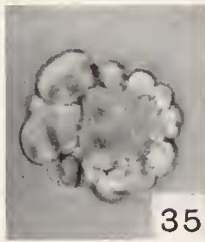
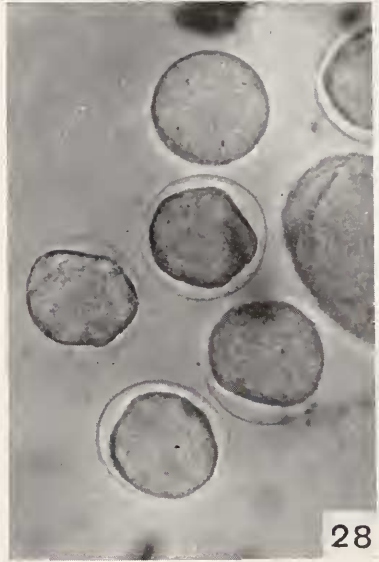
are activated, they throw off fertilization membranes widely separated from the surface of the egg, leaving a large perivitelline space just as is characteristic of the normal fertilized egg of this species (Photograph 27). The membranes are frequently thrown off while in the hypertonic solution and the surface of the egg then becomes irregular or crenate (Photograph 28). On return to sea water, these eggs swell and the perivitelline space is often completely obliterated; the fertilization membrane is frequently broken by further swelling. A large monaster forms three or four hours after activation and after another hour or so there may be two, three or more asters. Cleavage planes have been observed coming in around the asters, resulting in two-, three-, or four-cell stages (Photographs 29, 30, 31). No regular succession of cleavages has been observed, such as occur in *Arbacia*. The egg seems somewhat labile and cleavage planes appear and disappear. These eggs later break up into a number of small cells, becoming progressively smaller and more numerous, often while still within the fertilization membrane, resembling a blastula. Photographs 31, 32 and 33 are successive stages of the same egg showing the breaking up, following normal cleavage planes (Photograph 31) which had been observed to come in between three large asters. Photographs 34, 35 and 36 are successive stages of another egg. Many of these blastulæ occur as well as other blastulæ in which there are no cell boundaries; these may be filled with asters. The blastulæ emerge from the fertilization membranes in quite typical fashion on the day after activation, leaving the ruptured empty membranes (Photograph 37) and they may live for several days. The characteristic breaking up of the non-nucleate half-egg might be looked upon as stages in degeneration rather than as stages in development. But an exactly similar process takes place also

PHOTOGRAPHS 26-37

Parechinus microtuberculatus. Parthenogenetic merogones.

- PHOTOGRAPH 26. Non-nucleate lower halves, just broken off by centrifuging.
 PHOTOGRAPH 27. Ten minutes after activation, to show fertilization membranes.
 PHOTOGRAPH 28. Non-nucleate halves still in hypertonic sea water for 1/2 hour.
 PHOTOGRAPH 29. Beginning two-cell stage, still in hypertonic sea water for three hours.
 PHOTOGRAPH 30. Two-cell stage, seven hours after activation.
 PHOTOGRAPH 31. Three-cell stage, four hours after activation; three large asters were plainly visible but do not show in the photograph.
 PHOTOGRAPH 32. Same egg as 31, five hours later.
 PHOTOGRAPH 33. Same egg, one hour later (ten hours after activation).
 PHOTOGRAPH 34. Another egg, twenty-two hours after activation.
 PHOTOGRAPH 35. Same egg, five hours later.
 PHOTOGRAPH 36. Same egg, six hours later (thirty-three hours after activation).
 PHOTOGRAPH 37. Non-nucleate egg emerging from fertilization membrane, twenty hours after activation.

PLATE III



in the whole egg leading to normal blastulæ as described below. The similarity may be readily seen by comparing Photographs 34, 35 and 36 with Photographs 39, 40 and 42.

Haplones

Normal uncentrifuged eggs of *Parechinus microtuberculatus* were treated with hypertonic sea water for twenty-five minutes, then transferred to sea water. Fertilization membranes formed and after some hours the nuclei enlarged as they normally do previous to breaking down. In Photograph 38, taken seven and a half hours after activation, the enlarged nuclei may be seen in the two eggs to the left, and in the right-hand egg, the nuclear membrane has just broken (it was observed to break) and the cell has become somewhat amoeboid. Fifteen minutes later, this egg has begun to break up into a number of irregular cells (Photograph 39) and the process has continued further in Photograph 40, taken fifteen minutes later. The process continues as is shown in Photographs 41 and 42, until a blastula is formed, often quite normal in appearance, consisting of many cells of the same size. After some hours the blastulæ break through the fertilization membranes and become free-swimming, with cilia. Some of these seem quite regular and normal but some are abnormal consisting of cells of varying sizes. Many normal gastrulæ later developed, presumably (not actually observed) from the blastulæ which had developed in this irregular fashion (Photograph 43). A comparison of Photographs 34 with 39, 35 with 40, and 36 with 42 will show the similarity of the breaking up of the non-nucleate egg with the process in the nucleate parthenogenetic egg which gives rise to some quite normal-looking swimmers.

Some centrifuged whole eggs treated with the parthenogenetic agent cleaved similarly to such eggs after fertilization. In one set of

PHOTOGRAPHS 38-46

Parechinus microtuberculatus. Haplones.

PHOTOGRAPH 38. Normal eggs, 7½ hours after activation. Nuclear membrane had just broken in egg to right.

PHOTOGRAPH 39. Same eggs, 25 minutes later; egg breaking up irregularly.

PHOTOGRAPH 40. Same eggs, 15 minutes later.

PHOTOGRAPH 41. Similar eggs, 7½ hours after activation.

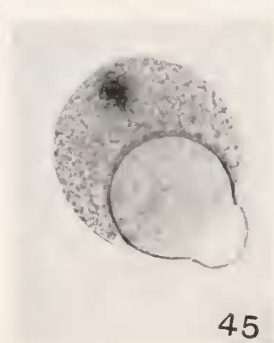
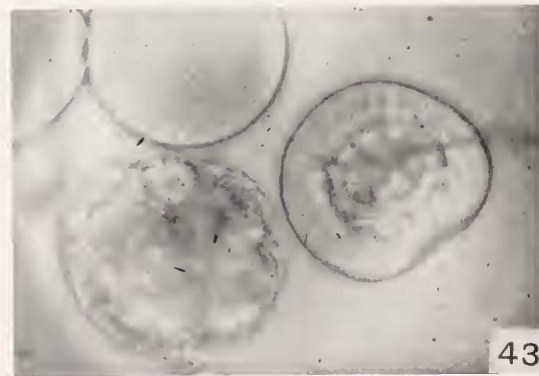
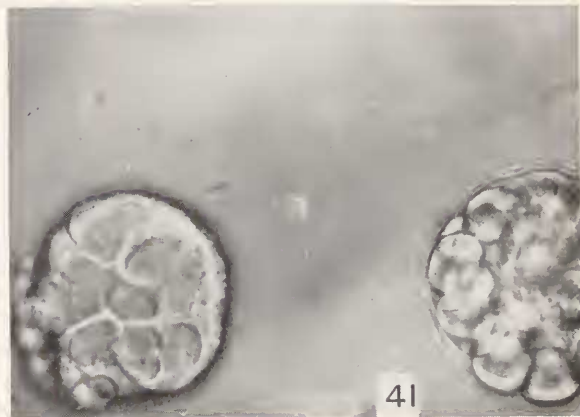
PHOTOGRAPH 42. Similar eggs, 7½ hours after activation. Note perfect blastula.

PHOTOGRAPH 43. Similar eggs, 24 hours after activation. Note perfect gastrula.

PHOTOGRAPH 44. Centrifuged egg; cell division, 15 minutes after activation, while nucleus is still intact.

PHOTOGRAPH 45. Similar eggs, 5 hours later; upper part cytolized.

PHOTOGRAPH 46. Fertilized merogone, 2 hours after fertilization.



parthenogenetic centrifuged eggs, however, a cleavage plane came in fifteen minutes after activation while the nucleus was still intact (Photograph 44). This came across the short axis of the egg, producing the same two-cell stage characteristic of the elongate *Arbacia* egg, both fertilized and parthenogenetic (Cf. Photograph 44 with 22, and with 30, 31 and 36 of my 1936 paper). This is another instance of cleavage planes coming in independently of any nuclear changes and emphasizes the fact that the cleavage of an egg and the nuclear changes usually accompanying it are quite separate phenomena. Later on, the upper part of these eggs cytolized, leaving the lower part with mitochondrial granules, intact (Photograph 45).

The fertilized merogones in many cases cleaved quite regularly. Photograph 46 is an eight-cell stage showing nuclei.

PARACENTROTUS LIVIDUS

Parthenogenetic Merogones

The development of the non-nucleate fractions of *Paracentrotus* after activation is exactly like that of *Parechinus* described above. The fertilization membrane is thrown off, leaving a large perivitelline space characteristic of the normal fertilized egg of this species. A conspicuous large aster is formed (Photograph 47) followed by two or more smaller asters. A few early cleavages have been observed (Photograph 48) but the cleavage planes come and go and then the egg breaks up into a number of cells becoming progressively smaller and more numerous, just as in *Parechinus*, giving rise to a blastula-like embryo (Photographs 49, 50).

PHOTOGRAPHS 47-58

PHOTOGRAPHS 47-51. *Paracentrotus lividus*. Photographs 52-58. *Sphaerechinus granularis*.

PHOTOGRAPH 47. *Paracentrotus lividus*. Non-nucleate half, 1½ hours after activation. Note large monaster.

PHOTOGRAPH 48. Beginning 2-cell stage, 4 hours after activation.

PHOTOGRAPH 49. Same egg as 47, 2½ hours after activation; egg fragmenting.

PHOTOGRAPH 50. Same egg 1/2 hour later.

PHOTOGRAPH 51. Parthenogenetic centrifuged whole egg, tripartite. Note aster in the stalk and many asters in upper part.

PHOTOGRAPH 52. *Sphaerechinus granularis*. Non-nucleate half, 2 hours after activation.

PHOTOGRAPH 53. Same egg, 3 hours later.

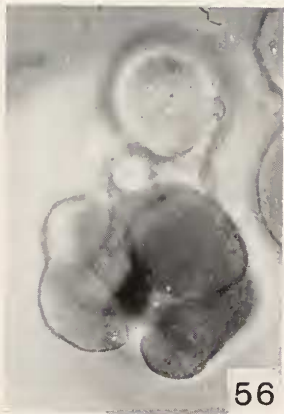
PHOTOGRAPH 54. Same egg, 1/2 hour later.

PHOTOGRAPH 55. Same egg, 1 hour later.

PHOTOGRAPH 56. Parthenogenetic centrifuged whole egg, 5 hours after activation.

PHOTOGRAPHS 57, 58. Cleavage of parthenogenetic centrifuged whole eggs while nucleus is still intact; 1 hour after activation.

PLATE V



Haplones

In the normal egg and in the centrifuged whole egg treated with hypertonic solutions, asters frequently appear in the protoplasm while the nucleus is still intact. Attention has previously been called (1933) to the peculiar tripartite eggs which develop after fertilization of a centrifuged egg, giving rise to three embryos. The middle portion of these eggs is simply the thickened stalk between the two half-eggs when incompletely broken apart. In a similar parthenogenetic egg, the fertilization membrane forms also around the stalk and a monaster appears in the stalk, even before the nucleus in the upper half has broken down. This is shown in Photograph 51 in which the upper part is filled with many asters (light areas in the photograph). Later on, the upper nucleate part of the centrifuged egg becomes cytolized, while the lower part may remain intact and even cleave. The cleavage of the lower part may also take place while the nucleus is intact in the upper part.

Swimmers have been obtained from parthenogenetic normal eggs but not from centrifuged whole eggs or the white nucleate halves after parthenogenetic reagents.

Good cleavages of the fertilized merogones have been obtained. Owing to the long period of centrifuging (thirty minutes) necessary for breaking apart the *Paracentrotus* egg, and the consequent heating up of the machine and the eggs, investigation of this species has been limited.

SPIHAERECHINUS GRANULARIS

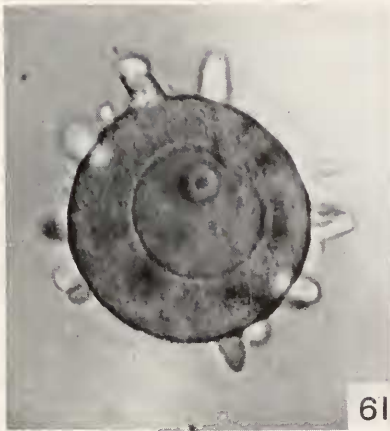
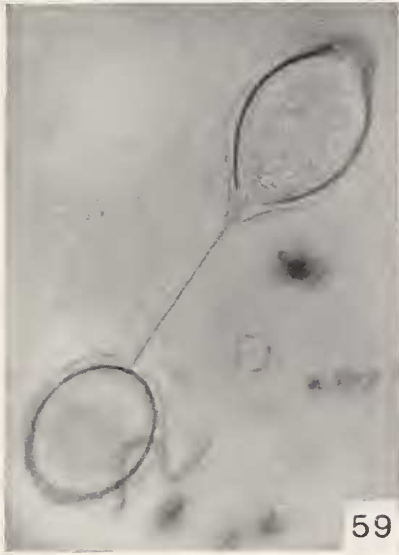
Parthenogenetic Merogones

The non-nucleate half of the *Sphaerechinus* egg, although larger in proportion than that of the other eggs studied and containing both mitochondria and yolk granules, does not develop as well. Fertilization membranes well separated from the surface, as is characteristic of the normal egg, are thrown off, often while still in the hypertonic solution. The egg then becomes amœboid, and fragments irregularly into a number of pieces. The irregularity of this process may be seen from Photographs 52-55 which are consecutive pictures of the same egg.

Haplones

The normal egg and the centrifuged whole egg after being subjected to parthenogenetic agents throw off fertilization membranes but do not go far in development. The centrifuged whole egg usually becomes

PLATE VI



PHOTOGRAPHS 59-62

Immature eggs of *Parechinus microtuberculatus*.

PHOTOGRAPH 59. Centrifuged egg, almost broken apart. Note thin layer of material over both parts and over connecting strand.

PHOTOGRAPH 60. Change of shape and breaking up of normal immature egg, one hour after hypertonic sea water.

PHOTOGRAPH 61. Normal immature egg in sea water showing protrusions wherever sperm have hit the surface.

PHOTOGRAPH 62. Immature egg after hypertonic sea water and return to sea water with sperm; no protrusions on the surface.

amœboid and breaks into a number of fragments just as the non-nucleate fraction does (Photograph 56).

A peculiar phenomenon was observed in the centrifuged whole eggs and the white (nucleate) halves kept for a long time (one hour) in the hypertonic solution. The nucleus became elliptical, the long axis of the ellipse always parallel with the planes of stratification. As it later moved down through the clear layer it moved with its long axis in any direction. The ellipse almost immediately rounded out into a sphere when transferred to sea water. The cause and meaning of the ellipse is not known. An elliptical nucleus has been observed also in the centrifuged eggs of *Arbacia punctulata* after fertilization, but here it seems to occur at a definite stage in development, just prior to its breaking down (See Fig. 28 of my 1932 paper).

A cleavage plane in the parthenogenetic centrifuged egg frequently comes in across the short axis of the egg (but not usually at its narrowest part) while the nucleus is still intact (Photographs 57, 58) just as in *Parechinus*, showing again that a cleavage plane may be independent of nuclear changes.

The fertilized merogones cleave and develop quite normally, as previously described (1933).

Immature Eggs

Since the immature egg differs from the mature in having a large amount of material in the germinal vesicle, which mixes with the egg substance on maturation, it would be of great interest to get the non-nucleate portion of an immature egg to develop. This has been tried, and although not successful, some interesting phenomena have been observed.

(1) Immature eggs incompletely broken apart by centrifugal force were put in a hypertonic solution. While still in the solution, a thin layer formed on the outside of both the nucleate and non-nucleate parts and also on the connecting strand (Photograph 59). No fertilization membrane was formed, but the layer of material resembled the ectoplasmic layer characteristic of mature eggs activated either by fertilization or by parthenogenetic agents.

(2) When normal uncentrifuged immature eggs were treated with a hypertonic solution, they became elongate, with the germinal vesicle at one end; the other end pinched off a small piece. Subsequently this lower piece divided into two and later into many parts (Photograph 60). The same result was obtained with centrifuged immature eggs. Though this is not a typical cleavage, the process is similar and indicates some sort of change in the surface similar to that occurring during a true cleavage of a mature egg.

(3) The fact that the surface of immature eggs is definitely changed by the parthenogenetic agent is shown by their reaction to sperm. The normal immature egg forms characteristic protrusions at the surface wherever the sperm hit (Photograph 61); these resemble enlarged fertilization cones. Immature eggs which have been in hypertonic sea water and are then put back into normal sea water and sperm added, do not form these protrusions or react in any way to the sperm (Photograph 62).

DISCUSSION

Although a further investigation of parthenogenetic merogony in four other species of sea urchin has not resulted in obtaining further development after the blastula stage, it has shown that the possibility of activating non-nucleate parts of eggs is fairly general. It may be that eggs will go only as far as the blastula stage without nuclei, and that nuclear material is necessary for differentiation, perhaps furnishing both chemical substances and genes.

All the non-nucleate parts will throw off a membrane, which is characteristic of the normal fertilized egg of its species, and they will all form an ectoplasmic layer and asters. The cleavage and early development does not seem to be dependent on any particular type of granule, for any type,—yolk, pigment or mitochondria, may be lacking in the different species and the egg still cleave. Cleavage is not dependent on a nucleus, for it may take place when it is completely absent or when it is present but intact and apparently inert. Nor is it dependent upon the presence of a spindle and asters as these may be centrifuged to another part of the cell and the cleavage plane come in without relation to them, as shown in a previous paper (1935). Cleavage seems to be dependent rather on the physical and chemical state of the protoplasm itself and of its surface layer. An orderly succession of cleavages is not necessary for the production of a many-celled organism. The cytoplasm can break up irregularly into fragments both when nuclear material is present and when it is not, and yet give rise to a blastula quite similar to that obtained by successive cleavages. The lability of cleavage planes and their disappearance and reappearance is characteristic of some of these non-nucleate fractions, just as was found for normal eggs deprived of oxygen (E. B. Harvey, 1927). Cleavage, budding and fragmentation seem to be different expressions of the same state of cytoplasm or of the cell surface, or both, in its effort to subdivide itself into smaller entities. Whatever the cell with a nucleus has been observed to do in this respect, the same cell without a nucleus has been observed to do, at least for a certain period. At

the time of cleavage, a tendency for the egg to become amœboid seems to be an expression of the physical state of the surface, just as the appearance of asters is usually associated with the state of the interior protoplasm at this time.

SUMMARY

1. The stratification, by centrifugal force, of the eggs of the four species of sea urchin occurring at Naples is described.

2. The non-nucleate parts of the eggs of all four species can be activated by parthenogenetic agents. They throw off fertilization membranes characteristic of the species, and begin to develop.

3. Development of the parthenogenetic merogones of *Arbacia pustulosa* is similar to that of *Arbacia punctulata*.

4. The parthenogenetic merogones of *Parechinus* and *Paracentrotus* develop by a process of progressive fragmentation inside the fertilization membrane, similar to that occurring in the parthenogenetic normal egg which leads to normal free-swimming blastulæ.

5. Cleavage may take place in whole eggs while the nucleus is still intact.

6. Certain changes take place in the immature egg similar to those of the mature egg when treated with parthenogenetic agents.

7. No further development of eggs without nuclei has yet been obtained beyond the blastula.

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